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Complementation of Vaccinia Virus Deleted of the E3L Gene by Mutants of E3L

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Vaccinia virus devoid of its E3L gene is sensitive to treatment of RK-13 cells with interferon-α and fails to replicate or form plaques in HeLa cells. In order to determine function of the E3L gene, vaccinia virus recombinants were constructed by inserting mutant E3L genes or a gene coding for an alternative dsRNA-binding protein into virus deleted of its wild type E3L gene. Those viruses that expressed proteins that retained dsRNA binding activity were resistant to the effects of interferon in RK-13 cells and could replicate in HeLa cells. Recombinant viruses that expressed E3L mutant proteins which were unable to bind to dsRNA were interferon sensitive in RK-13 cells and could not replicate in HeLa cells. In addition, a virus that expressed a mutant E3L protein previously characterized as having a low binding affinity for dsRNA exhibited an intermediate phenotype: it was interferon resistant in RK-13 cells but could not replicate in HeLa cells. This work suggests that the E3L gene of vaccinia virus functions primarily as a dsRNA-binding protein in allowing resistance to interferon and in promoting replication in HeLa cells.

INTRODUCTION

Replication of vaccinia virus (VV) is resistant to pretreatment of a number of cells with type-I interferon. The interferon resistance phenotype of vaccinia virus is associated with the E3L gene (Beattie *et al.,* 1995a,b). The E3L gene encodes two proteins, p25 and p20, both of which bind to double stranded RNA (dsRNA) (Chang *et al.,* 1992; Yuwen *et al.,* 1993). E3L inhibits activation of two interferon-induced, dsRNA-activated enzymes, the protein kinase, PKR, and 2′,5′-oligoadenylate synthetase (Beattie *et al.,* 1995a). Activation of PKR involves autophosphorylation and subsequent phosphorylation of several substrates, including the α subunit of eukaryotic protein synthesis initiation factor 2 (eIF-2) (Samuel, 1979). These events lead to a block in protein synthesis initiation, and therefore to impairment of translation. Activation of 2′,5′-oligoadenylate synthetase leads to synthesis of short 2′,5′-linked oligomers of ATP (Kerr and Brown, 1978) that can activate a constitutive RNase, RNase L, which can degrade both viral and cellular RNAs (Floyd-Smith *et al.,* 1981).

Inhibition of PKR and 2′,5′-oligoadenylate synthetase is thought to be due to the dsRNA binding activity of the E3L products. The E3L gene has been sequenced and the protein product has been characterized (Chang *et al.,* 1992; Yuwen *et al.,* 1993). The protein contains a dsRNA binding domain (dsRBD) that is found in other proteins known to bind to dsRNA, such as PKR (Galabru and Hovanessian, 1987), the double stranded adenosine deaminase (Kim *et al.,* 1994), human TAR RNA binding protein (TRBP) (Park *et al.,* 1994); *Xenopus* rbpa (Bass *et al.,* 1994); *Drosophila melanogaster* staufen gene product (Ferrandon *et al.,* 1994), the pac1 gene product of the fission yeast *Schizosaccaromyces pompbe* (Rotondo *et al.,* 1995); *Escherichia coli* RNase III (Court, 1993), and group C rotavirus p8 (Langland *et al.,* 1994). For several proteins, including the E3L gene products, the dsRBD has been shown to be sufficient for binding to dsRNA. In fact, the group C rotavirus p8 protein, at 69 amino acids, consists of little more than a dsRBD (Langland *et al.,* 1994).

Vaccinia virus deleted of the E3L gene, VVΔE3L, is interferon sensitive in RK-13 cells (Chang *et al.,* 1995) and mouse L929 cells (Beattie *et al.,* 1995a) and has a restricted host range phenotype (Chang *et al.,* 1995). VVΔE3L can have its host range restored by transient transfection of plasmids which supply the wt E3L product *in trans* (Chang *et al.,* 1995). Plasmids which code for mutants of E3L that do not bind dsRNA fail to rescue replication of VVΔE3L (Chang *et al.,* 1995), suggesting that the E3L gene products function to promote replication in a wide variety of host cells by binding to and sequestering dsRNA. The restricted host range and interferon sensitivity of E3L deletion mutants can also be at least partially rescued *in trans* by the reovirus σ3 protein (Beattie *et al.,* 1995a), which has been shown to bind to

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dsRNA (Huismans and Joklik, 1976). Since σ3 has little amino acid sequence similarity to the E3L products, these results again argue that the mechanism of action of E3L is through its dsRNA binding activity.

The transient transfection results have demonstrated a correlation between ability of the E3L product to bind to dsRNA and unrestricted host range phenotype (Chang *et al.,* 1995). However, the ability of E3L mutant proteins to rescue the interferon sensitivity of VVΔE3L cannot be shown *in trans.* Interferon sensitivity of VVΔE3L cannot be tested in HeLa cells, which are easily transfected at high efficiency, because this virus does not replicate in HeLa cells (Chang *et al.,* 1995). VVΔE3L can replicate in RK-13 cells (Chang *et al.,* 1995), but these cells have a very low transfection frequency, which prevents detectable rescue of virus deleted for E3L from the effects of IFN treatment. In this report, various mutant E3L genes were recombined into VVΔE3L. Those resultant viruses which expressed functional dsRNA binding proteins were resistant to the effects of interferon in RK-13 cells and could replicate in HeLa cells. Recombinant viruses that expressed E3L mutant proteins which were unable to bind to dsRNA were interferon sensitive in RK-13 cells and could not replicate in HeLa cells. Thus, confirming the results of the transient transfections, a correlation exists between the viral expression of a dsRNA binding protein and the viral host range phenotype. This correlation is extended here to include restoration of interferon resistance.

MATERIALS AND METHODS

Cells and viruses

HeLa cells were maintained as monolayers in Eagle's minimum essential medium (MEM) supplemented with 50 μg/ml gentamicin sulfate and 5% fetal bovine serum (Hyclone). RK-13 cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in MEM supplemented with 1$\times$ nonessential amino acids (GIBCO), 50 μg/ml gentamicin sulfate, and 5% fetal bovine serum (Hyclone). All cell lines were maintained in a 37°, 5% CO_2 environment.

Vaccinia virus VC-2 (Copenhagen strain), designated in this paper as wtVV, was the parent for all viruses used in this study.

Plasmid constructs for *in vivo* recombination

The pMPE3LΔGPT 748 recombination vector was kindly supplied by James Tartaglia (Virogenetics). This plasmid contains left and right E3L flanking regions, including the E3L promoter, adjacent to the *E. coli gpt* gene, which codes for resistence to mycophenolic acid. All variants of the E3L gene reconstructed into VVΔE3L were from VV, WR strain. The Δ83N mutant of E3L was originally constructed by Hwai-Wen Chang (Chang and Jacobs, 1993). The Δ83N E3L mutant was subcloned into pBSIIKS$^+$. This was done by cleaving pGEM-5T/Δ83N (Chang and Jacobs, 1993) with *Eco*RI and inserting it into previously *Eco*RI cut pBSIIKS$^+$. The Δ83N E3L was determined to be in the T7 orientation in pBSIIKS$^+$. Next, the Δ83N E3L gene was removed from the pBSIIKS$^+$ vector via *Hin*cII and *Bam*HI digestion and subcloned into pMPE3LΔGPT 748 that had been previously cut with *Pst*I, treated with T4 DNA polymerase and cleaved with *Bam*HI. Inserted Δ83N was confirmed via *Hin*dIII/*Bam*HI digestion.

The Δ37N mutation was created by PCR of pBS-E3L with the following oligonucleotides: 5′-AGGCCTGCAGATGGAGAAGCGAGAAGTT-3′ (initiator codon underlined) and 5′-TCGCGAATTCTCAGAATCTAATGATGAC-3′. The PCR product was cleaved with *Pst*I and *Eco*RI and cloned into pBSIIKS$^+$. The altered gene was then subcloned into pMPE3LΔGPT 748. The pBSIIKS$^+$/Δ37N construct was cleaved with *Hin*cII and a phosphorylated *Bam*HI linker was inserted into the *Hin*cII site. Subsequently, the construct containing the linker (pBSIIKS$^+$/Δ37N) and pMPE3LΔGPT 748 were cleaved with *Bam*HI, and the E3L mutant gene was inserted into the *Bam*HI site of pMPE3LΔGPT 748. Orientation of the E3L genes was determined by *Pst*I digestion.

Construction of VVE3LΔ7C, VVE3LΔ26C, VVE3LG164V, VVΔE3L/p8, and VVΔE3L/S4 has been previously described (Kibler *et al.,* 1997). The E3L gene was reconstructed into VVΔE3L after subcloning the E3L coding region from pBS-E3L into pMPE3LΔGPTMCS using *Bam*HI and *Hin*dIII.

In vivo recombination of genes into VVΔE3L

Insertion of genes into VVΔE3L, selection of recombinants, and growth of virus stocks was performed as previously described, using either $CaPO_4$ precipitation (Baier *et al.,* 1993) or lipofection (Kibler *et al.,* 1997) to transfect DNA into cells. Briefly, cells infected with VVΔE3L (which contains a *lacZ* gene in the E3L locus) were transfected with the indicated plasmid. After a single cycle of replication, released virus was plated onto RK-13 cells in the presence of mycophenolic acid to select for viruses that had incorporated the plasmid through a single recombination event. After several rounds of plaque purification in the presence of mycophenolic acid, viruses were plated in the absence of mycophenolic acid to allow resolution of plasmid sequences by homologous recombination. Viruses which had incorporated the desired gene in place of *lacZ* were identified as forming clear plaques after staining with X-gal. Constructs were verified by PCR analysis for deletion mutants and by sequencing for point mutants.

IFN sensitivity/plaque reduction assays

RK-13 cells (seeded in six-well, 10 cm^2/well dishes) were pretreated for 24 h with 0, 0.1, 1.0, 10, 100, and

1000 Units/ml of rabbit interferon (Lee Biomolecular). Each well of cells was then infected with 0.1 ml of MEM containing 2% FCS and containing 50-100 PFUs and the virus was adsorbed for 1 h and overlaid with liquid medium. The infected cells were incubated for 48 h at 37°. Plaques were visualized by aspirating the medium and staining the monolayers with 0.1% crystal violet in 20% ethanol for 5 min at room temperature (Earl and Moss, 1991). All assays were repeated a minimum of three times.

Single step growth curve of recombinant viruses

Confluent monolayers of rabbit RK-13 cells were either treated with 200 U/ml of rabbit interferon-α for 22 h or were mock-treated. Cells were infected with various recombinants of VV at an m.o.i. of 1. Infected cells were harvested at 1, 6, 12, 18, and 24 or 30 h postinfection. Each time point was titered via standard plaque assays on monolayers of RK-13 cells and stained with crystal violet as described above.

RESULTS

The VV E3L gene is necessary for interferon resistance (Beattie *et al.,* 1995a,b) and to allow replication in a wide range of host cells, including HeLa cells (Chang *et al.,* 1995). In order to better define the function of the E3L gene we have replaced the wt E3L gene with genes coding for various mutants of E3L or with a gene coding for the porcine group C NSP3 p8 protein. Porcine p8 is the smallest known natural dsRNA-binding protein and is thought to consist of little more than a dsRBD (Langland *et al.,* 1994). Mutants of E3L included proteins that bind to dsRNA with wt affinity but which are altered in localization within the cell (VVE3LΔ83N; see Fig. 1A; Chang and Jacobs, 1993; Chang *et al.,* 1995), proteins that bind to dsRNA with wt affinity and localize normally within the cell (VVE3LΔ37N; data not shown), proteins with decreased affinity for dsRNA (VVE3LΔ7C; Chang and Jacobs, 1993), and proteins which fail to bind to dsRNA (VVE3LΔ26C and VVE3LG164V; Chang and Jacobs, 1993). In addition, as a control, we have inserted the full-length WR strain E3L gene into virus deleted for E3L (VVrE3L).

IFN sensitivity assays on vaccinia recombinants

To determine if mutants of the E3L gene could ameliorate the IFN sensitive phenotype of VVΔE3L, plaque reduction assays were performed in RK-13 cells as described under Materials and Methods. Results are shown in Fig. 2. Plaque formation by wtVV, as well as by recombinants that contained mutant E3L genes encoding functional dsRNA binding proteins in which the binding domain has not been altered (VVrE3L, VVE3LΔ37N, and VVE3LΔ83N) were resistant to interferon pretreatment

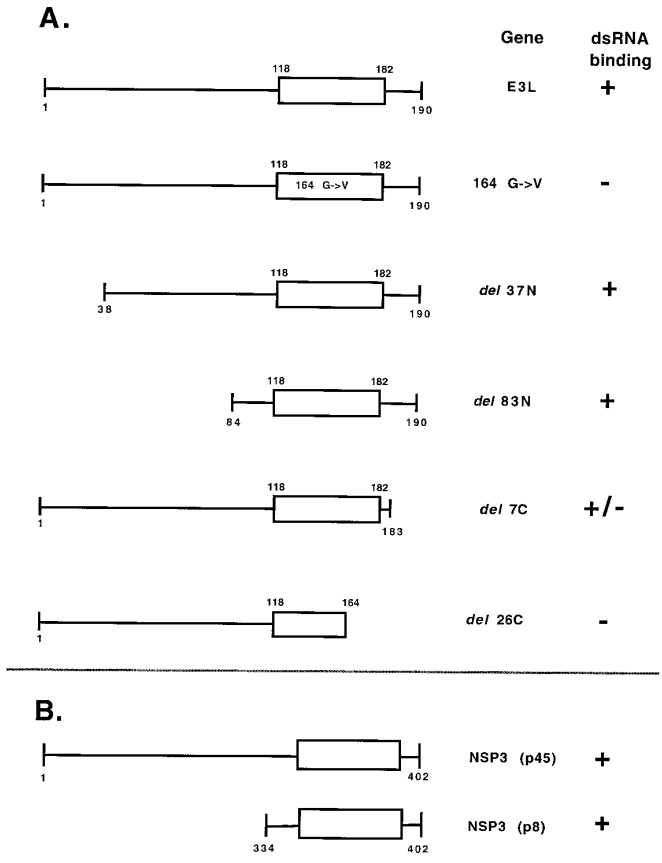

FIG. 1. Schematic summary of the dsRNA binding activity of the various proteins whose genes have been inserted into the E3L locus of VVΔE3L. The numbers indicate the amino acid residue within the protein. Boxes indicate the conserved dsRNA binding motif. (A) Mutants of E3L. (B) The group C rotavirus NSP3 gene.

with up to 1000 U/ml. The VVE3LΔ7C recombinant virus was also fully interferon resistant. Previous characterization of the Δ7C gene in the transient-transfection assay showed that it rescued VVΔE3L protein synthesis and replication poorly in HeLa cells (Chang *et al.,* 1995), presumably due to its reduced affinity to bind dsRNA (Chang and Jacobs, 1993). Virus containing the porcine group C rotavirus p8 gene had intermediate sensitivity to interferon when measured by a plaque reduction assay. Plaque formation by VVΔE3L/p8 was reduced 60–80% in cells treated with 100–1000 U/ml of interferon. Those viruses which contained E3L genes that were altered within the dsRNA binding domain rendering them unable to bind to dsRNA, VVE3LG164V and VVE3LΔ26 C, yielded results comparable to VVΔE3L.

Restoration of host range phenotype by vaccinia recombinants

Vaccinia virus deleted of its E3L gene cannot form plaques in HeLa cells (Chang *et al.,* 1995). We wanted to determine which mutants of E3L would restore plaque formation in HeLa cells. Therefore, HeLa cells were in-

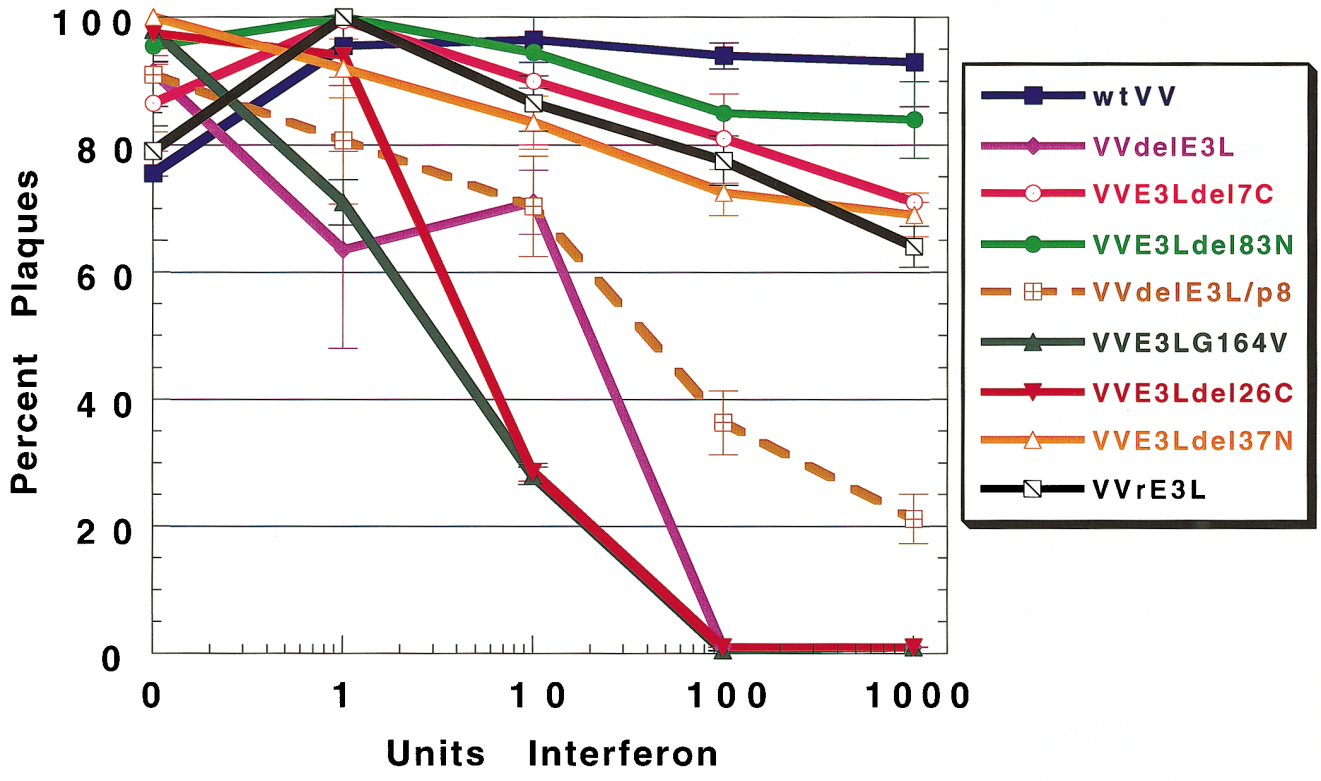

FIG. 2. Plaque reduction assays to determine interferon sensitivity of vaccinia virus recombinants were performed in RK-13 cells as described under Materials and Methods. The data are shown as percentage of the maximum number of plaques for each virus.

fected with the recombinant viruses and efficiency of plaquing in HeLa cells was analyzed relative to plaquing in RK-13 cells (Table 1). wtVV, VVrE3L, VVE3LΔ37N, and VVΔE3L/p8 were able to plaque in HeLa cells with the EOP ranging from 0.1 to 2.0. No plaques were observed with VVΔE3L, VVE3LG164V, or VVE3LΔ26C. These results correlate with the dsRNA binding phenotype. In addition, VVE3LΔ7C had an EOP of $<1.3 \times 10^{-5}$ and did not plaque in HeLa cells. This was somewhat surprising since VVE3LΔ7C was fully resistant to interferon treatment of RK-13 cells (Fig. 2) and since transient expression of Δ7C partially rescued replication of VVΔE3L (Chang *et al.,* 1995).

TABLE 1

Efficiency of Plaquing HeLa Cells

Virus	RK-13	HeLa	EOP
wtVV	3.15×10^9	9.4×10^8	0.45
VVΔE3L	2.60×10^8	$<1.00 \times 10^3$	$<1.30 \times 10^{-6}$
VVE3LΔ37N	3.50×10^8	7.05×10^8	2.00
VVE3LΔ83N	3.00×10^8	5.50×10^7	0.39
VVE3LG164V	1.70×10^7	$<1.00 \times 10^3$	$<5.60 \times 10^{-5}$
VVE3LΔ7C	7.70×10^7	$<1.00 \times 10^3$	$<1.30 \times 10^{-5}$
VVE3LΔ26C	2.00×10^7	$<1.00 \times 10^3$	$<5.00 \times 10^{-5}$
VVΔE3L/p8	2.90×10^8	5.90×10^7	0.18
VVrE3L	4.10×10^8	1.80×10^8	0.44

Note. Representative results of virus stocks titered in RK-13 or HeLa cells. The data are shown as mean plaque forming units per milliliter (PFU/ml). The relative efficiency of plaquing (HeLa/RK-13, EOP) is also shown.

Single step growth curve of vaccinia recombinants

Single step growth curves were performed in HeLa cells (Fig. 4) or in RK-13 cells in the absence or pretreatment of interferon (Fig. 3). All viruses were able to replicate with titers ranging from 2×10^7 PFU/ml to 1×10^8 PFU/ml in untreated RK-13 cells (Figs. 3A and 3C). In RK-13 cells pretreated with interferon, titers of VVΔE3L, VVE3LG164V, and VVE3LΔ26C were reduced 200-fold as compared to wtVV (Figs. 3B and 3C). Again, replication in the presence of interferon correlated with the dsRNA binding phenotype. wtVV, VVrE3L, VVE3LΔ83N, VVE3LΔ37N, and VVE3LΔ7C replication was fully resistant to treatment with interferon, in agreement with the results of plaque reduction assays (Fig. 2). Somewhat surprisingly, replication of VVΔE3L/p8 under single cycle conditions was completely resistant to pretreatment with interferon (Fig. 3C), in contrast to results from plaque reduction assays (Fig. 2).

Replication in HeLa cells under single cycle conditions gave results similar to those obtained under plaque assay conditions. Those viruses that did not express

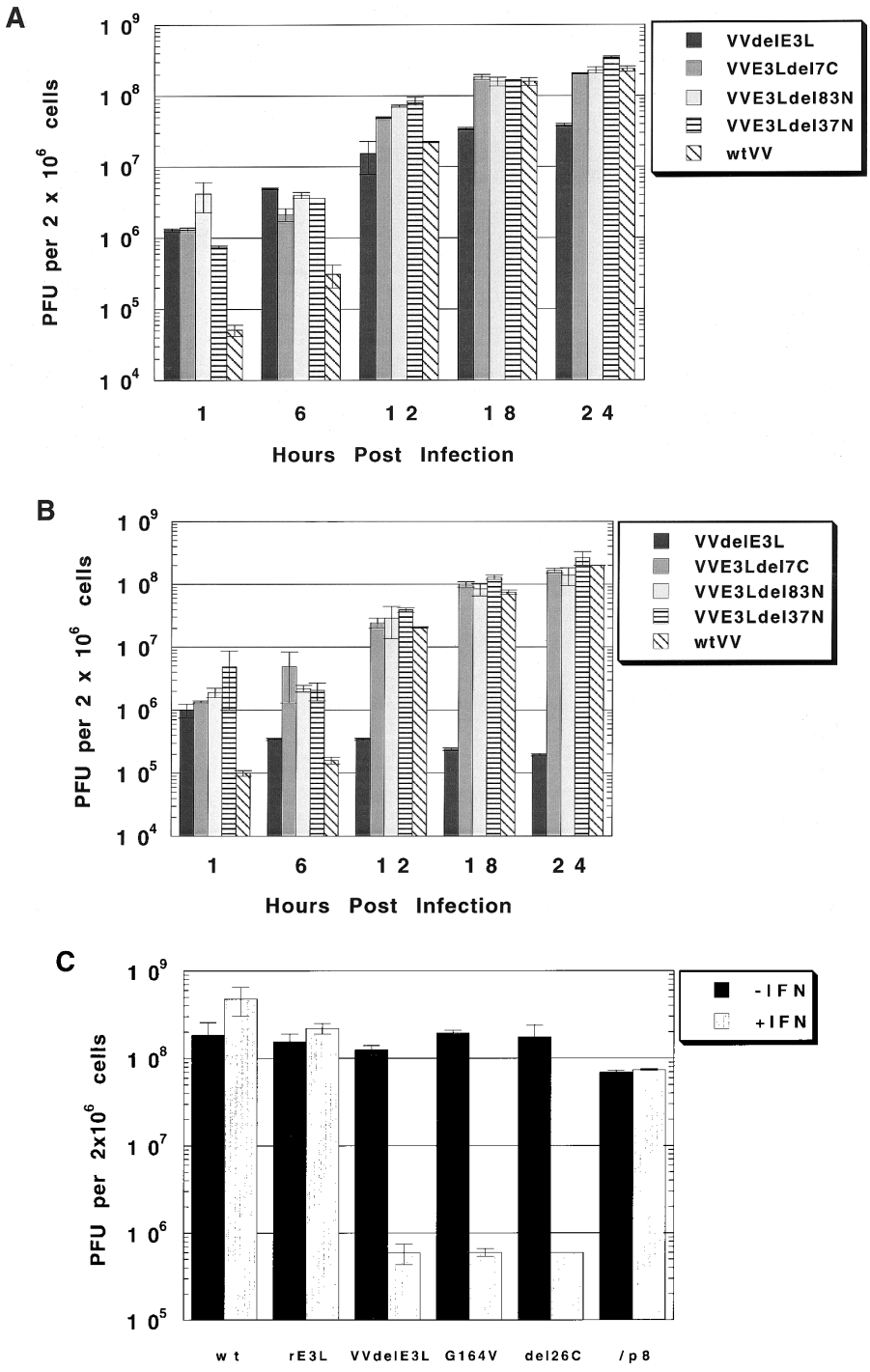

FIG. 3. Single step growth curves of recombinant viruses in RK-13 cells with (B and C as indicated) or without interferon (A and C as indicated) pretreatment. Cells were infected with an m.o.i. of 1 and each time point was titered in RK-13 cells via standard plaque assays and stained via crystal violet. (A and B) Viruses were harvested at 1, 6, 12, 18, and 24 h postinfection. (C) Viruses were harvested at 30 h postinfection.

functional dsRNA binding proteins, VVΔE3L, VVE3-LG164V, and VVE3LΔ26C, did not replicate in HeLa cells (Figs. 4A and 4B). wtVV, VVrE3L, VVE3LΔ83N, and VVE3LΔ37N yielded similar titers of $2-5 \times 10^8$ PFU/ml in HeLa cells (Figs. 4A and 4B). Yields of VVΔE3L/p8 were routinely two- to threefold lower than wtVV in both HeLa cells (Fig. 4B) and in RK-13 cells (Fig. 3C).

DISCUSSION

In this report, we demonstrate that products of the VV E3L gene which contain a functional dsRNA binding domain are necessary to mediate the interferon resistance phenotype of vaccinia virus and for replication of vaccinia virus in HeLa cells. We recombined several mu-

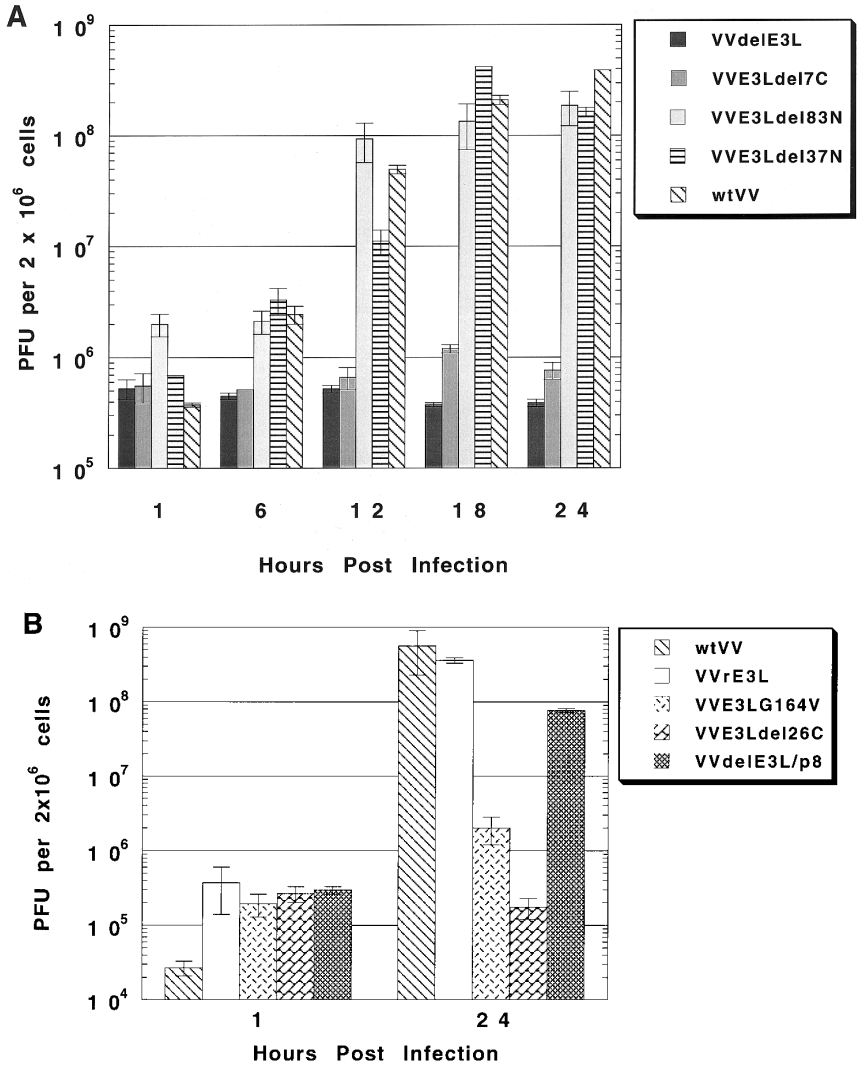

FIG. 4. Single step growth curves of recombinant viruses in HeLa cells. Cells were infected with an m.o.i. of 1 and each time point was titered in RK-13 cells via standard plaque assays and stained via crystal violet. In A, viruses were harvested at 1, 6, 12, 18, and 24 h postinfection. In B, viruses were harvested at 1 and 24 h postinfection.

tants of the E3L gene into a virus deleted of its E3L gene, which is interferon sensitive and unable to replicate in HeLa cells. We were then able to ask which recombinant viruses were resistant to the effects of interferon. Recombinants containing an E3L gene with deletions of 37 or 83 amino acids from its N terminus were interferon resistant and able to replicate in HeLa cells. These mutations have no affect on dsRNA binding activity or PKR inhibition (Chang and Jacobs, 1993). Thus, to the limit of the assays described in this paper, the N-terminal 44% of the E3L gene is completely dispensible for interferon resistance and to promote replication in HeLa cells. Since the protein encoded by this gene does not migrate to the nucleus of transfected (Chang *et al.,* 1995) or infected cells (unpublished observations), these results suggest that nuclear localization is unimportant for these phenotypes. This result is somewhat surprising in that the N-terminus of E3L is well conserved between vaccinia virus, variola virus (Shchelkunov *et al.,* 1993) and ectromelia virus (M. Buller, pers. commun.). A 25-kDa protein that cross-reacted with anti-E3L serum was also detected in cowpox- and rabbitpox-infected cells (unpublished observations). Perhaps the N-terminus of E3L is important for replication in whole animals or for replication under different conditions than those described in this paper. Virus containing Δ83N does induce apoptosis in infected cells, but only at very late times (36 h) postinfection (unpublished observations).

Virus containing Δ7C, which binds to dsRNA with low affinity (Chang and Jacobs, 1993), had an intermediate phenotype. VVE3LΔ7C was fully resistant to treatment of RK-13 cells with interferon, but failed to replicate even in untreated HeLa cells. This intermediate phenotype is consistent with what is known about Δ7C protein and about HeLa cells and RK-13 cells. HeLa cells contain constitutively high levels of activatable PKR. RK-13 cells

contain very low levels of activatable PKR, even after treatment with interferon (Kibler *et al.,* 1997). Δ7C protein, with its low affinity for dsRNA is presumably able to overcome the low levels of activatable PKR in RK-13 cells, but not the constitutively high levels found in HeLa cells.

Recombinant viruses containing E3L gene products with a deletion (VVE3LΔ26C) or point mutation (VVE3LG164V) within the carboxyl terminus of the protein that destroys dsRNA binding activity (Chang and Jacobs, 1993) were sensitive to the effects of interferon and unable to replicate in HeLa cells. These results, along with the results described in the previous paragraphs, suggest that E3L primarily functions as a dsRNA-binding protein in promoting resistance to interferon and replication in HeLa cells. This conclusion is supported by virus containing the gene encoding the porcine group C rotavirus p8 protein. At 69 amino acids this protein is the smallest known natural dsRNA-binding protein (Langland *et al.,* 1994). Since the entire dsRBD is thought to be 69 amino acids long, this protein likely does not contain any functional domains other than a dsRBD. Virus containing this protein is quite resistant to the effects of interferon and plaques normally and replicates to reasonable titers in HeLa cells. We cannot rule out a minor role for some other function of E3L, since VVΔE3L/p8 was somewhat sensitive to interferon treatment in plaque reduction assays, and did not replicate to quite as high titers as wtVV in either HeLa or RK-13 cells.

The VV E3L gene is also necessary to prevent induction of apoptosis in infected cells (Lee and Esteban, 1994; Kibler *et al.,* 1997). We have previously shown that E3L is primarily functioning as a dsRNA-binding protein in inhibiting induction of apoptosis (Kibler *et al.,* 1997). To a large extent our results suggest that failure to replicate, either in interferon-treated RK-13 cells or in untreated HeLa cells correlates with induction of apoptosis. However, this is not absolutely the case since VVE3LΔ83N does induce apoptosis in HeLa cells at late times postinfection (unpublished observations), yet replicates normally. It is likely that apoptosis is being induced in these cells after the virus has productively replicated and thus does not interfere with virus replication. We have also isolated an as yet uncharacterized virus that contains a mutation in the E3L gene, which fully supports replication in HeLa cells and interferon-treated RK-13 cells, even though it does not prevent induction of apoptosis (unpublished observations).

In summary, dsRNA binding of the E3L gene products, p25 and p20, is indispensible for countering the antiviral effects established by the host.

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REFERENCES

Baier, L. J., Shors, T., Shors, S. T., and Jacobs, B. L. (1993). The mouse antiphosphotyrosine kinase, TIK, is indistinguishable from the double-stranded RNA-dependent, interferon-induced protein kinase, PKR. *Nucleic Acids Res.* 21, 4830–4835.

Bass, B. L., Hurst, S. R., and Singer, J. D. (1994). Binding properties of newly synthesized Xenopus proteins containing dsRNA-binding motifs. *Curr. Biol.* 4, 301–314.

Beattie, E., Denzler, K. L., Tartaglia, J., Perkus, M. E., Paoletti, E., and Jacobs, B. L. (1995a). Reversal of the interferon-sensitive phenotype of a vaccinia virus lacking E3L by expression of the reovirus S4 gene. *J. Virol.* 69, 499–505.

Beattie, E., Paoletti, E., and Tartaglia, J. (1995b). Distinct patterns of IFN sensitivity observed in cells infected with vaccinia K3L- and E3L-mutant viruses. *Virology* 210(2), 254–263.

Chang, H.-W., Watson, J. C., and Jacobs, B. L. (1992). The E3L gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* 89, 4825–4829.

Chang, H.-W., and Jacobs, B. L. (1993). Identification of a conserved motif that is necessary for binding of the vaccinia virus E3L gene products to double-stranded RNA. *Virology* 194(2), 537–547.

Chang, H.-W., Uribe, L. H., and Jacobs, B. L. (1995). Rescue of vaccinia virus deleted for the E3L gene by mutants of E3L. *J. Virol.* 69, 6605–6608.

Court, D. (1993). RNA processing and degradation by RNase III. *In* "Control of Messenger RNA Stability" (J. G. Belasco and G. Brawerman, Eds.), pp. 71–116. Academic Press, San Diego.

Faulkner, F. G., and Moss, B. (1990). Transient dominant selection of recombinant vaccinia viruses. *J. Virol.* 64, 3108–3111.

Ferrandon, D., Elphick, L., Nusslein-Volhard, C., and St. Johnston, D. (1994). Staufen protein associates with the 3′UTR of bicoid mRNA to form particles that move in a microtubule-dependent manner. *Cell* 79(7), 1221–1232.

Floyd-Smith, G., Slattery, E., and Lengyel, P. (1981). Interferon action: RNA cleavage pattern of a (2′-5′)oligoadenylate-dependent endonuclease. *Science* 212, 1030–1032.

Galabru, J., and Hovanessian, A. G. (1987). Autophosphorylation of the protein kinase dependent on double-stranded RNA. *J. Biol. Chem.* 262, 15538–15544.

Ho, C. K., and Shuman, S. (1996). Mutational analysis of the vaccinia virus E3 binding to double-stranded RNA. *J. Virol.* 70, 2611–2614.

Ho, C. K., and Shuman, S. (1996). Physical and functional characterization of the double-stranded RNA binding protein encoded by the vaccinia virus E3 gene. *Virology* 217, 272–284.

Hovanessian, A. G. (1989). The double stranded RNA-activated protein kinase induced by interferon: dsRNA-PK. *J. Interferon Res.* 9, 641–647.

Huismans, H., and Joklik, W. K. (1976). Reovirus-coded polypeptides in infected cells: Isolation of two native monomeric polypeptides with affinity for single-stranded and double-stranded RNA, respectively. *Virology* 70, 411–424.

Jacobs, B. L., and Langland, J. O. (1996). When two strands are better than one: The mediators and modulators of the cellular responses to double-stranded RNA. *Virology* 219, 339–349.

Kaempfer, R., Israili, R., Rosen, H., Knoller, S., Zilberstein, A., Schmidt, A., and Revel, M. (1979). Reversal of the interferon-induced block of protein synthesis by purified preparations of eucaryotic initiation factor 2. *Virology* 99, 170–173.

Kalvakolanu, D. V., and Borden, E. C. (1996). An overview of the inter-

feron system: Signal transduction and mechanisms of action. *Cancer Invest.* 14(1), 25–53.

Kerr, I. M., and Brown, R. E. (1978). pppA2′p5′A: An inhibitor of protein synthesis synthesised with an enzyme from interferon-treated cells. *Proc. Natl. Acad. Sci. USA* 75, 256–260.

Kharrat, A., Macias, M. J., Gibson, T. J., Nilges, M., and Pastore, A. (1995). Structure of the dsRNA binding domain of E. coli RNase III. *EMBO J.* 14(14), 3572–3584.

Kibler, K. V., Shors, T., Perkins, K. B., Zeman, C. C., Banaszak, M. P., Biesterfeldt, J., Langland, J. O., and Jacobs, B. L. (1997). Double-stranded RNA is a trigger for apoptosis in vaccinia virus-infected cells. *J. Virol.* 71, 1992–2003.

Kim, U., Wang, Y., Sanford, T., Zeng, Y., and Nishikura, K. (1994). Molecular cloning of cDNA for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing. *Proc. Natl. Acad. Sci. USA* 91, 11457–11461.

Langland, J. O., and Jacobs, B. L. (1992). Cytosolic double-stranded RNA-dependent protein kinase is likely a dimer of partially phosphorylated Mr = 66,000 subunits. *J. Biol. Chem.* 267, 10729–10736.

Langland, J. O., Pettiford, S., Jiang, B., and Jacobs, B. L. (1994). Products of the porcine group C rotavirus NSP3 gene bind specifically to double-stranded RNA and inhibit activation of the interferon-induced protein kinase PKR. *J. Virol.* 68, 3821–3829.

Lee, S. B., and Esteban, M. (1994). The interferon-induced double-stranded RNA-activated protein kinase induces apoptosis. *Virology* 199, 491–496.

Mazzara, G. P., Destree, A., and Mahr, A. (1993). Generation and analysis of vaccinia virus recombinants. *In* "Methods in Enzymology" (R. Wu, Ed.), pp. 557–581. Academic Press, San Diego.

Park, H. M., Davies, M. V., Langland, J. O., Chang, H. W., Nam, Y. S., Tartaglia, J., Paoletti, E., Jacobs, B. L., Kaufman, R. J., and Venkatesan, S. (1994). TAR RNA binding protein is an inhibitor of the interferon-induced protein kinase PKR. *Proc. Natl. Acad. Sci. USA* 91, 4713–4717.

Rotondo, G., Gillespie, M., and Frendewey, D. (1995). Rescue of the fission yeast snRNA synthesis mutant snm1 by overexpression of the double-strand-specific Pac1 ribonuclease. *Mol. Gen. Genet.* 247(6), 698–708.

Saccomanno, L., and Bass, B. L. (1994). The cytoplasm of Xenopus oocytes contains a factor that protects double-stranded RNA from adenosine-to-inosine modification. *Mol. Cell. Biol.* 14(8), 5425–5432.

Samuel, C. E. (1979). Mechanisms of interferon action: Phosphorylation of protein synthesis initiation factor eIF-2 in interferon-treated human cells by a ribosome-associated kinase possessing site-specificity similar to hemin-regulated rabbit reticulocyte kinase. *Proc. Natl. Acad. Sci. USA* 76, 600–604.

Samuel, C. E. (1993). The eIF-2a protein kinases, regulators of translation in eukaryotes from yeasts to humans. *J. Biol. Chem.* 268, 7603–7606.

Shchelkunov, S. N., Blinov, V. M., and Sandakhchiev, L. S. (1993). Genes of variola and vaccinia viruses necessary to overcome the host protective mechanisms. *FEBS Lett.* 319, 80–83.

Shors, T., and Jacobs, B. L. (1997). Complementation of deletion of the vaccinia virus E3L gene by the *Escherichia coli* RNase III gene. *Virology* 227, 77–87.

Watson, J., Chang, H. W., and Jacobs, B. L. (1991). Characterization of a vaccinia encoded double-stranded RNA binding protein that may be involved in inhibition of the double-stranded RNA-dependent protein kinase. *Virology* 185, 206–216.

Wek, R. C. (1994). eIF-2 kinases: Regulators of general and gene specific translation initiation. *Trends Biochem. Sci.* 19, 491–496.

Younger, J., Thacore, H., and Kelly, M. (1972). Sensitivity of ribonucleic acid and deoxyribonucleic acid viruses to different species of interferon in cell culture. *J. Virol.* 10, 171–180.

Yuwen, H., Cox, J. H., Yewdell, J. W., Bennink, J. R., and Moss, B. (1993). Nuclear localization of a double-stranded RNA-binding protein encoded by the vaccinia virus E3L gene. *Virology* 195, 732–744.